Cell Division: Aurora B Illuminates a Checkpoint Pathway

Separating mitotic error correction, chromosome biorientation and the spindle assembly checkpoint (SAC) is complicated by their interconnected relationships. New research finds that Aurora B kinase, which drives error correction and promotes biorientation, also directly regulates the SAC.

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To the casual scientific passer-by, the spindle assembly checkpoint (SAC) pathway may look foreboding. This feeling is not without merit. After all, those that venture down this path quickly encounter an entangled molecular network that has been difficult to experimentally unravel. The most ensnaring problems have stemmed from efforts to address the relative contributions of tension and attachment to error correction and the SAC (reviewed in [1,2]). At the heart of the debate lies a kinase that has many roles during mitosis — Aurora B. It is generally accepted that Aurora B mediates error correction; however, the question of whether Aurora B directly contributes to SAC signaling has been difficult to resolve. Three recent studies have advanced the discussion by demonstrating that Aurora B is, indeed, a checkpoint kinase [3–5].

The SAC promotes equal distribution of the genome between two daughter cells by preventing anaphase until every chromosome has become bioriented within the mitotic spindle (reviewed in [6]). When chromosomes are not bioriented, kinetochore-templated protein complexes act in concert with checkpoint kinases to produce a 'wait-anaphase' signal. The molecular output of this signaling network is a soluble anaphase-promoting complex/cyclosome (APC/C) inhibitor called the mitotic checkpoint complex (MCC), which includes the checkpoint proteins Mad2, BubR1 and Bub3, and the APC/C co-regulator Cdc20. Two critical non-MCC checkpoint components include Mad1, the kinetochore-associated binding partner of Mad2, and the kinase Mps1. Both Mad1 and Mps1 localize to the kinetochores of improperly attached chromosomes. Kinetochore-bound Mad1–Mad2 (Kt-Mad1–Mad2) catalyzes the association of Mad2 with Cdc20 by converting the dynamic 'open' form of Mad2 (O-Mad2), which has low affinity for Cdc20, to 'closed' Mad2 bound to Cdc20 (C-Mad2–Cdc20), a complex that is competent to assemble into the MCC and inhibit the APC/C [7]. Mps1 is required for the localization of Kt-Mad1–Mad2 upon mitotic entry and the subsequent conversion of O-Mad2 to the inhibitory C-Mad2–Cdc20 complex [8–11]. Thus, there is no doubt that Mad1 and Mps1 are *bona fide* checkpoint regulators.

Debate, however, swirls around the checkpoint credentials of Aurora B kinase. Aurora B, which is required for correcting improper kinetochore–microtubule interactions, is hypothesized to destabilize non-bioriented attachments by phosphorylating attachment factors, like the Ndc80 complex, and reducing their affinity for microtubules [12] (and reviewed in [2,13]). By and large, researchers agree that Aurora B indirectly contributes to the SAC during error correction by creating unattached (or weakly attached) kinetochores that, in turn, stimulate production of the wait-anaphase signal (Figure 1). However, previous investigations into whether Aurora B directly impacts SAC signaling have yielded confounding results (reviewed in [2]). Three new studies have overcome earlier limitations by experimentally separating error correction from SAC signaling [3–5].

Both Santaguida *et al.* [4] and Saurin *et al.* [5] experimentally isolated error correction from checkpoint signaling by depolymerizing microtubules with nocodazole. This treatment causes an extended mitotic arrest that, since error correction is not possible in the absence of microtubules, is mediated exclusively by the wait-anaphase signal generated by the SAC. Both groups used the nocodazole-induced mitotic arrest as an assay to dissect the direct contributions of Aurora B, Mps1 and the Ndc80 complex (Hec1) to the SAC pathway. The localization and subsequent activation of kinetochore-associated Mps1 (Kt-Mps1), which normally occurred in nocodazole-treated cells, was found by Saurin *et al.* [5] to be delayed by either chemical inhibition of Aurora B or depletion of the Ndc80 complex. Delaying the activation of Kt-Mps1 resulted in reduced levels of kinetochore-associated Mad2 in early mitosis and, as a result, delayed SAC establishment upon mitotic entry. While a wait-anaphase signal was eventually established when either Aurora B activity or the Ndc80 complex were individually depleted, the SAC was completely bypassed when Aurora B and Ndc80 were inhibited together or in combination with Mps1 inhibition. Tethering Mps1 to the kinetochore by fusing it to the outer kinetochore component Mis12 rescued the effects of inhibiting Aurora B and Ndc80. Thus, Saurin *et al.* [5] conclude that Aurora B, Ndc80 and Mps1 synergize to establish the SAC. They further postulate that the primary checkpoint role of Aurora B is limited to early mitosis when it acts in concert with the Ndc80 complex to enrich Mps1 at kinetochores.

Santaguida *et al.* [4] agree that Aurora B, the Ndc80 complex and Mps1 act in synergy to impart checkpoint signaling. However, they found that addition of higher concentrations of Aurora B inhibitors, which still specifically inhibited Aurora B kinase activity, dramatically reduced the duration of the nocodazole-induced mitotic arrest without impacting kinetochore assembly. Titrations of reversine (an Mps1 inhibitor) [11] and hesperadin (an Aurora B inhibitor) [14] combined with simulated dose-response analyses strongly suggest that researchers have been partially inhibiting Aurora B activity in previous investigations. For example, the most commonly used concentrations of the two Aurora B inhibitors ZM447439 [15] (2 μM) and hesperadin (100 nM) yield ~50% and <90% inhibition, respectively. Given that complete abolition of the SAC has historically required highly penetrant depletions of checkpoint regulators [16,17], it is likely that the contradictory findings from previous Aurora B studies can be explained by partial inhibition of its kinase activity.

Saurin *et al.* [5] and Santaguida *et al.* [4] both convincingly demonstrated that Aurora B acts upstream of Kt-Mad1–Mad2. However, their

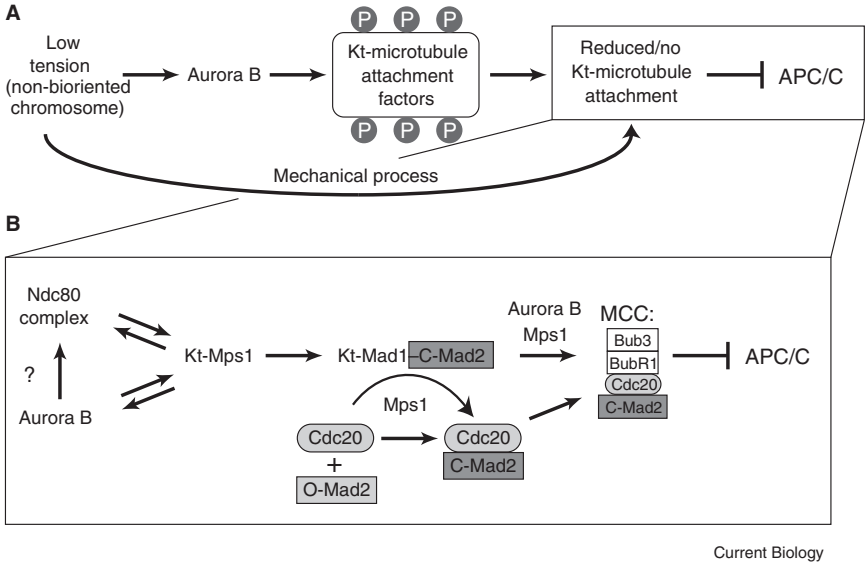

Figure 1. Aurora B kinase acts at multiple points along the SAC pathway.

(A) Aurora B indirectly contributes to SAC signaling via its well established role in error correction by phosphorylating Kt (kinetochore)-microtubule attachment factors and reducing their affinity for microtubules at non-bioriented chromosomes that are under low tension. Unattached and/or weakly attached kinetochores created by Aurora B generate a wait-anaphase signal that inhibits the APC/C. Tension also mechanically stabilizes Kt-microtubule attachments independent of Aurora B [18]. (B) Aurora B directly regulates SAC signaling by acting upstream and downstream of Kt-Mad1–Mad2. Aurora B, together with the Ndc80 complex, localizes and activates Kt-Mps1. While phosphorylation of Ndc80 by Aurora B is important for error correction, it is not known (?) if this also contributes to Kt-Mps1 recruitment. Feedback mechanisms (double arrows) are likely to play a role in this process since there is evidence that Mps1 phosphorylates and interacts with Ndc80 [19] and also regulates Aurora B activity [20]. Kt-Mps1 is required for the kinetochore localization of Mad1, which tightly interacts with the 'closed' form of Mad2 (C-Mad2). Kt-Mad1–C-Mad2 together with Mps1 stimulate the conversion of open Mad2 (O-Mad2) to C-Mad2 bound to Cdc20 [8,10], an essential prerequisite to MCC assembly. Finally, Aurora B acts downstream of Kt-Mad1–C-Mad2 by promoting MCC assembly.

findings diverged when they evaluated the contribution of Aurora B further downstream. While Saurin and colleagues [5] found that inhibiting Aurora B in cells that were first arrested in nocodazole did not dramatically alter the checkpoint response, Santaguida *et al.* [4] observed that a more potent inhibition of Aurora B in nocodazole-arrested cells (<6 hour) led to a premature mitotic exit. Luckily, experiments by Maltonado and Kapoor have helped to fill this gap [3]. To examine the contribution of factors that act downstream of Kt-Mad1–Mad2, Maltonado and Kapoor [3] constitutively targeted Mad1 to the kinetochore by fusing it to Mis12. Expression of the constitutive Kt-Mad1 protein in human tissue culture cells led to the persistent recruitment of Mad2 to kinetochores. Furthermore, constitutively localizing Kt-Mad1–Mad2 was sufficient to stimulate production of the wait-anaphase signal even when chromosomes were properly attached and aligned — a condition that normally satisfies the SAC. Interestingly, the outer kinetochore environment was essential for this effect as targeting Mad1–Mad2 to chromosome arms failed to illicit a checkpoint arrest. Not surprisingly, BubR1, which is an MCC component, and Mps1, which is required for C-Mad2–Cdc20 complex formation, were both required for the experimentally induced arrest. More surprising was the fact that Aurora B kinase activity was also required for the Kt-Mad1–Mad2-mediated arrest, suggesting that Aurora B acts downstream of Kt-Mad1–Mad2. This notion is supported by the observations of Santaguida *et al.* [4] that potent inhibition of Aurora B kinase activity bypassed an established mitotic arrest, mislocalized BubR1 and Bub1 from kinetochores in nocodazole-treated cells (an effect that was not observed following Mps1 inhibition), reduced BubR1 and Bub1 phosphorylation, and disrupted BubR1 incorporation into the MCC.

It now appears that, like Mps1, Aurora B acts at multiple points along the SAC pathway (Figure 1). In addition to its established role in creating unattached kinetochores during error correction, the evidence suggests that Aurora B, in conjunction with synergistic partners, acts both upstream and downstream of Kt-Mad1–Mad2: upstream, with the Ndc80 complex, to localize and activate Kt-Mps1, and downstream, with Mps1, to promote phosphorylation of BubR1 and its association with the MCC. Given the degree to which Aurora B activity had to be inhibited to bypass the nocodazole arrest, the fact that it acts in synergy with multiple checkpoint regulators and that Aurora B is required for both Kt-Mad1–Mad2 association and BubR1–MCC interactions, it is likely that Aurora B kinase plays a central role in one of the most defining yet poorly understood characteristics of the SAC — wait-anaphase signal amplification. Determining the full extent to which Aurora B regulates the SAC will certainly require future investigation but the composite picture painted by the three works discussed here is clear — Aurora B kinase activity is directly involved in checkpoint signaling. With this knowledge and new assays in hand, the field can continue cutting through entanglements along a SAC pathway that now looks much less foreboding.

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